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EXPERIMENTAL MEASLES BY INOCULATION OF MONKEYS, GUINEA-PIGS AND RABBITS WITH A GREEN-PRODUCING DIPLOCOCCUS

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In earlier papers one of us[1] has described a small gram-positive diplococcus, which was isolated from the blood, eye, nose, throat and sputum of patients with measles, during the preeruptive and early eruptive stage of the disease. The organism was cultivated only in anaerobic cultures from the blood, but it generally grew aerobically in the second generation. The diplococcus could be cultivated readily from the respiratory tract, producing small green colonies on aerobic blood-agar plates. When first isolated and small these cocci generally passed through a tested Berkefeld N filter. Diplococci corresponding morphologically to those in cultures could be demonstrated often in large numbers in smear preparations from the tonsils, nose, sputum and eye.

The blood of patients with measles showed a distinct increase in specific agglutinins and opsonins for this diplococcus from the third to the eighth day following the appearance of the eruption. Complement fixing bodies were present only to a slight extent as the symptoms subsided.

Blood of rabbits, immunized with 4 strains from the blood, eye, ear and nose of patients with measles, showed specific opsonic and agglutinating power not only for the strains used in immunization, but for others from the blood, nose, throat and sputum.

This diplocccus was pathogenic for the rabbit, dog, mouse and rat but not for the guinea-pig, when injected intravenously or intraperitoneally, but no rashes or symptoms of measles were observed in the earlier experiments.

At the height of the attack of measles, gram-positive bacilli and other organisms were sometimes isolated from the blood, but as they were not found in the preeruptive stage, when the blood is known to

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[1] Tunnicliff, R.: Jour. Am. Med. Assn., 1917, 68, p. 1028; Jour. Infect. Dis., 1918, 22, p. 402; Jour. Am. Med. Assn., 1918, 71, p. 104. Tunnicliff, R., and Brown, M. W.: Jour. Infect. Dis., 1918, 23, p. 572. Tunnicliff, R.: Ibid., 1919, 24, p. 76; p. 181.

be infective, and no opsonins, agglutinins or complement-fixing bodies (unpublished) could be demonstrated for these organisms, they were not considered of any etiologic significance.

The successful intratracheal inoculation of monkeys with measles virus by Blake and Trask,[2] led to the use of this method, with one exception, in the following experiments: Virus was obtained by washing the nose and throat of patients with measles in the preeruptive or early eruptive stages, with salt solution, or by carefully swabbing the nose and throat and washing the swabs in salt solution, or in a few instances incubating them in ascitic dextrose broth for 24 hours, centrifuging and suspending the sediment in salt solution. The cocci were isolated from the original swabs on aerobic goat blood agar plates. Single colonies were inoculated into ascitic dextrose broth and on blood agar slants. The ascitic dextrose broth cultures were centrifuged and the cocci suspended in salt solution. When the growth on blood agar was used it was washed off in salt solution. The cocci were small, gram-positive, round or slightly elongated diplococci, bile insoluble, fermenting lactose and salicin but not mannite or inulin. They grew in pairs or short chains in broth and produced small green colonies on blood-agar plates.

MONKEYS

Exper. 1.—April, 1918. A rhesus monkey was injected intravenously with the sediment of 100 c c horse serum dextrose broth culture of coccus isolated from blood of a patient with measles 6 hours before the appearance of the eruption. This was the third generation of the organism. The monkey was very ill for one day and slightly so for a few days following the injection. It then remained well for 10 days, when it became less active. On this day the face was flushed, but no eruption was observed either in the mouth or on the skin. There was no rise in temperature. A distinct leukopenia was present from the fourth to the thirteenth day. This reaction was considered due to the coccus because two other monkeys inoculated with equally large numbers of spirilla isolated from the blood of patients with measles produced no such change.

Exper. 2.—March 26, 1921. A monkey was injected intratracheally with 4 c c of salt solution suspension of the growth of 4 colonies of cocci from the nose and throat of a patient with measles with Koplik spots, 24 hours before the appearance of the eruption. When inoculated the face of the monkey was flushed, the eyes were somewhat injected, and bright red macules were present at the outer angles of the eyes. The mucous membrane of the mouth was rather rough and red. The animal remained well and active for 8 days, when it became quiet. On the eleventh day the mucous membrane of the mouth was more congested and on the twelfth day it appeared red and rough with blister-like whitish spots, suggestive of Koplik spots, especially at the angles of the jaw; the mucous membrane of the mouth remained rough and red throughout the experiment. There was diarrhea on the twelfth day. On the following day the monkey was listless, the eyes somewhat watery and the face more flushed than usual. The temperature was slightly elevated on the tenth day and remained so most of the time for 10 days, during which period there was a decided fall in the number of leukocytes. Anaerobic blood cultures on the fifteenth day were negative. Cultures from the mouth on the tenth day showed green-producing diplococci which were much smaller than those normally present and which appeared similar to those injected.

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[2] Jour. Exper. Med., 1921, 33, p. 385.

This monkey was reinoculated intratracheally with 6 c c of nasopharyngeal washings from a measles patient with Koplik spots, 24 hours before the appearance of the eruption. This injection was followed by no rise in temperature, no exanthem, and no symptoms of measles, except a drop in the number of leukocytes from the fifth to the tenth day following this injection.

Exper. 3.—May 13, 1921. An intratracheal injection was made with a monkey with 8 c c of salt solution suspension of growth of four colonies from throat of patient with Koplik spots, one day before the appearance of measles eruption. When injected there was a marked erythema on the face of the monkey, with a macular eruption around the eyes, which were injected, the inner canthus being swollen and red. Mucous membrane of mouth was bleeding, rough and red. Four days after the injection the monkey was less active and lost its appetite. The mucous membrane on the buccal surfaces were red and rough with whitish spots, suggestive of Koplik spots, but they persisted throughout the experiment. On the fifteenth day the face was more flushed than usual especially around the nose. One bright red papule, 1 mm. in diameter, appeared on the left cheek. It disappeared the following day. On the sixteenth day the face was more flushed, there was slight photophobia and another small bright pink papule appeared on the chin disappearing the following day and leaving some desquamation. On the seventeenth day there were also some copper colored macular patches on the chest with scales. There was a slight elevation of temperature on the sixteenth day, when the leukocytes dropped to their lowest point. There was a leukopenia from the tenth to the nineteenth day. A blood culture made on the sixteenth day was negative. Cultures from the mouth were made several times, but no green-producing diplococci were found which corresponded morpholgically to those injected. This animal was later inoculated with the growth of 3 tubes of ascitic dextrose broth cultures of the same culture as used originally, but no rise in temperature, no leukopenia, no eruption and no symptoms were produced.

Exper. 4.—May 13, 1921. An intratracheal injection was made into a monkey with 7 c c of salt solution suspension of growth of cocci from the throat of a child at the onset of the measles eruption. At the time of injection the skin of the face and the mucous membranes of the mouth of the monkey were pale. The animal remained well for 8 days when it appeared quiet. The following day the inner canthus of the eyes was congested. On the tenth day a small discrete bright red spot appeared on the left buccal mucous membrane. Two days later the mucous membrane of the lips was congested. On the fifteenth day there was a slightly red granular patch on the left side of the buccal mucous membrane, which appeared on the right side the following day. This condition disappeared by the twenty-first day. Cultures from these areas showed almost pure cultures of green-producing diplococci, which were small, round or slightly elongated. They disappeared with the congestion. On the eighteenth day a bright pink papule, about 2 mm. in diameter, appeared at the angle of the right eye and a bright pink macule on the left. They disappeared on pressure. The following day another small red papule appeared behind the right ear and a few pinkish macules on the cheek. The next day the eruption had disappeared, except around the eyes where it persisted 3 days, leaving a few scales. Another bright pink papule appeared on the neck on the twenty-first day. On the seventeenth and eighteenth days there was a slight rise in temperature. There was a marked leukopenia from the ninth to the twenty-first day. Anaerobic blood cultures made on the eighteenth day were negative.

The papule which appeared on the twenty-first day was excised, fixed in Zenker's fluid and stained with alum-hematoxylin and eosin followed by Gram's stain. The sections showed a slight proliferative and exudative reaction about the capillaries and small veins in the upper part of the corium. The endothelial cells of the capillaries were swollen, and there was an accumulation of endothelial leukocytes around them. A few of these cells were in mitosis. A few eosinophils and polymorphonuclear leukocytes and lymphocytes were present around the vessels. Similar changes were seen around the sebaceous glands. In one place there was slight exudation of serum and infiltration of the epidermis with endothelial leukocytes. Some of these cells in the lower part of the epidermis were in mitosis. No necrosis was observed in the epidermis, perhaps due to the age of the papule. The sections were compared with sections of skin from early human measles and corresponded closely. The changes in the corium appeared similar to those described by Ewing[3] and Mallory and Medlar[4] in human measles and by Blake and Trask[2] in measles in monkeys.

This monkey was reinoculated with 8 c c of washings from the nose and throat of a patient with measles with Koplik spots, at the onset of the rash. Thirteen days later the temperature rose from 101.8 to 103 F. and remained above 102 for 2 days. On the thirteenth day one bright red spot appeared on the left buccal mucous membrane. A few pink macules were observed on the chin and a yellowish maculopapular eruption on the abdomen. On the head, arms and abdomen was an uncolored papular eruption. The animal did not appear sick. The following day a slightly pinkish granular rash was seen on the lower labial mucous membrane and 3 bright red spots on the mucous membrane of the upper lip. The pinkish maculopapular exanthem extended around the mouth and on the thighs. The next day the mouth appeared normal. The eruption had extended to the upper part of the tail but was fading on the face, leaving fine scales. On the seventeenth day the rash was all gone. Desquamation in large flakes was observed on the arms and abdomen. No appreciable leukopenia occurred after this inoculation.

A small green-producing diplococcus was isolated from the mouth on the fourteenth day.

Sections of skin removed on this day showed the same changes seen in the section of skin removed following the injection of diplococci, except that there was a larger number of eosinophils and polymorphonuclear leukocytes around the vessels and one vesicle in the epidermis, infiltrated with polymorphonuclear leukocytes.

These experiments indicated that the diplococcus isolated from the blood and secretions of early cases of measles produced a mild reaction in monkeys; in one case protecting against a subsequent inoculation of virus, in a second against another injection of cocci. The last experiment suggests that the attack was too mild to protect against a possible overwhelming dose of virus. According to Kerr,[5] relapses in cases of measles are exceedingly rare. He has seen only one case in which a typical attack was followed 7 to 8 days later by a repetition of the disease with equally classical symptoms. Osler[6] says that relapses are occasonally seen, "the symptoms recurring at intervals from 10 to 40 days, but it is not always easy to say whether there may not have been a new infection from without."

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[3] Jour. Infect. Dis., 1909, 6, p. 1.

[4] Jour. Med. Research, 1920, 41, p. 327.

[5] Infectious Diseases, p. 52.

GUINEA-PIGS

Ten guinea-pigs were insufflated intratracheally with from 1 to 2 c c of material through a small rubber catheter, as described by Rosenow.[7] The hair on the chest and abdomen was removed with clippers. Two animals were inoculated with measles virus, 5 with the second and third generations of the green-producing diplococci isolated from this material and 3 with hemolytic streptococci from scarlet fever. One guinea-pig inoculated with virus showed no leukopenia, but a rise of temperature beginning on the seventh day. One injected with diplococci from this material died on the second day with bronchopneumonia. The guinea-pigs inoculated with throat virus and the 4 with measles cocci showed a drop in the number of leukocytes on the fourth and fifth days, coincident with or followed by a rise in temperature of 2 to 3 degrees. This reaction rarely persisted more than one day. An occasional animal coughed, showed some coryza, conjunctivitis and injection of the mucous membrane of the mouth, but Koplik spots and eruptions were observed in none. Green-producing diplococci similar to those injected were isolated from the lung of the guinea-pig with bronchopneumonia and from one with coryza.

Two guinea-pigs injected with hemolytic streptococci showed a decided rise in the number of leukocytes on the fourth and fifth days, without a rise in temperature; the third guinea-pig died on the seventh day with a great reduction in the number of leukocytes and a slight rise in temperature.

These experiments suggest that the virus, as well as the diplococcus from measles, produces in guinea-pigs a leukopenia accompanied by a rise in temperature with no marked respiratory symptoms and no eruptions.

Since these experiments were made Duval and D'Aunoy[8] have shown that guinea-pigs react specifically to intracardiac injections of defibrinated blood from cases of human measles. They observed a definite and constant rise in temperature and a coincident decrease in the total number of leukocytes after an incubation period of 9 to 12 days. A typical exanthem was not noted. Coryza and injections of the buccal mucous membrane occurred in some animals but were so inconstant as not to be included as part of the specific reactions. An intense hemorrhagic nephritis was a constant pathologic finding.

The difference in the incubation period between their guinea-pigs and ours may be due to the source of the material and method of injection.

RABBITS

Nevin and Bittman[9] and Grund[10] appear to have demonstrated that rabbits are susceptible to the virus of measles and within rather wide limits give a characteristic syndrome. Nevin and Bittman used blood from human patients and Grund the nasopharyngeal secretions from the same patients. They found the temperature and blood counts unreliable. Conjunctivitis and inflammation of the upper respiratory passages in varying degree of severity were noted. They observed eruptions both in the mouth and in the skin after an incubation period of from 2-8 days. Typical Koplik spots were found by Nevin and Bittman in about 33% of the rabbits, in 15% by Grund; eruptions were observed 100% by Nevin and 75% by Grund. The exanthem, according to Nevin, was in no instance typical of measles. It varied from a slight flush over the chest and in the axillary region to general redness extending to the groin. Grund observed either a diffuse punctate erythema and following the erythema, sometimes occurring without it, a maculopapular rash which faded after from 2-4 days, and left pigmentation persisting until desquamation began.

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[6] Practice of Medicine, Ed. 9, p. 348.

[7] Jour. Am. Med. Assn., 1919, 72, p. 1608.

[8] Jour. Exper. Med., 1922, 35, p. 257.

[9] Jour. Infect. Dis., 1921, 29, p. 429.

[10] Ibid., 1922, 30, p. 86.

We have injected 9 rabbits intratracheally with measles virus, 22 with the green-producing diplococci from measles and 15 with control organisms. The hair of the chest and abdomen and in some, o fthe back was removed by shaving or by the use of depilatory. Nine of the control cultures—7 staphylococci, 1 hemolyzing streptococcus and 1 producing large moist green colonies—were isolated from the same plates from which the green-producing diplococci from measles were isolated, with the idea that if the virus was carried by bacteria on the plate it might be transferred as readily by one organism as by another. The other control cocci were 4 strains of Strep. viridans, one isolated from a measles patient as the rash was fading, two from the throat of a German measles patient, one from diphtheria, and two strains of hemolyzing streptococci from scarlet fever. All of the organisms injected were of the second generation unless otherwise stated.

Leukocyte counts were made on the first 4 rabbits injected, 2 with virus and 2 with the green-producing diplococci. As no appreciable change in the number of leukocytes was demonstrated, although good rashes were observed, the blood counts were discontinued. A rise in temperature above 104 F. was not constant, occurring in only a few rabbits, but when present it coincided with the appearance of the exanthem. A temperature of 103.8 F. is not infrequent in uninoculated rabbits. Conjunctivitis, coryza and reddening of the buccal mucous membranes were rarely more marked than in the uninoculated rabbits, but small, slightly elevated red spots with whitish centers, which we interpreted as Koplik spots, were observed. The centers were never bluish, but neither were those in the human cases from whom the material was obtained. These spots were seen in 42 per cent. of the rabbits injected with diplococci from measles but were never observed in rabbits inoculated with virus; this may have been due to the smaller number of rabbits so injected. Five spots were seen in 1 rabbit, 2 in another, but as a rule only one would occur on one side and sometimes another on the opposite side, the following day. They appeared in from 3 to 15 days after inoculation, generally about the seventh day, and persisted from 1-3 days. They were followed in from 1-5 days by the eruption which appeared from 7-18 days after injection, generally on the eleventh day. Exanthems were observed in 87% of the rabbits inoculated with virus, in 84% of those with diplococci from measles. One rabbit with Koplik spots did not show any eruption. The exanthem consisted of a diffuse erythema over the chest, abdomen and back, or of a pink maculopapular eruption of varying extent, with or without erythema, persisting from 1-6 days, generally about 3. The macules were never so red nor so large as in human measles. In some instances the exanthem was followed by pigmentation, generally by desquamation in fine or coarse scales. The redness and desquamation that followed shaving and the use of depilatory disappeared before the animals were injected.

The third, fourth, fifth and seventh generations of the measles diplococci on blood agar still produced Koplik -like spots and eruptions when injected into rabbits. The strain which produced lesions as late as the seventh generation was plated out twice in the interim.

Small pieces of skin were removed during the height of the eruption in three rabbits injected with diplococci from measles. The same changes were found as observed in the lesions in man and monkeys, except that there was a more marked infiltration around the vessels and a larger number of polymorphonuclear leukocytes and eosinophils and more mitoses. The blood vessels showed considerable distention, chiefly with red blood corpuscles, but often with polymorphonuclear leukocytes and eosinophils. There was also greater invasion of the epidermis. The lesions here showed an exudation of serum and endothelial leukocytes into the stratified epithelium, collecting in some places near the outer surface and producing small vesicles. Some of the endothelial cells were multiplying. No bacteria could be demonstrated in the skin sections.

Koplik-like spots were removed from 3 rabbits and found to correspond to the skin lesions except that the invasion of the epithelium was more marked. In one instance an erosion was observed. The microscopic pathology of these spots is similar to that of Koplik spots in man and monkeys.[2, 3, 4] Gram-positive coccus-like bodies in pairs were seen in 2 specimens outside and inside cells around some of the vessels. They appeared somewhat like the bodies described by Mallory and Medlar in the skin of patients with measles. However, it is very difficult to differentiate cocci from granules in such sections.

As pointed out by Grund, there are a fairly large number of refractory rabbits, only 1 animal out of 2 injected with the same material developing symptoms. In successfully inoculated animals there is a leakage from the nose. In 2 rabbits injected with measles diplococci in which there was no leakage, no symptoms occurred. Although 2 other rabbits receiving the same material reacted, they are included in the series as negative.

None of the rabbits injected with bacteria isolated from the same plate from which the green-producing diplococci from measles were isolated showed Koplik-like spots or eruptions. Four were later reinoculated with virus or measles diplococci and all developed exanthems, 1 Koplik spots. Two rabbits injected with hemolyzing streptococci, 1 from scarlet fever, showed an erythema the following day. Edema of the abdomen was observed in 1 injected with a Strep. viridans from diphtheria. In fact, none of the 15 control rabbits showed Koplik spots or rashes similar to those produced by the virus or diplococci from measles.

Considering that the diplococcus cultures as late as the seventh generation could produce a reaction in rabbits and that they were originally isolated from single colonies and that rabbits similarly inoculated with second generation cultures of staphylococci and green and hemolyzing streptococci from the same plates produced no symptoms, the chance of the selective transfer of the virus by only the green-producing diplococci appears small.

CULTIVATION EXPERIMENTS

The green-producing diplococci are not easily cultivated from the blood of successfully inoculated monkeys and rabbits. In the original work, blood cultures were chiefly made from children acutely ill, into mediums containing ascitic fluid or horse serum. In mild adult human cases they were found difficult to isolate, only two successful cultures being obtained from 20 patients. In one case, the patient had a bronchopneumonia on the day the rash appeared; in the other, the blood was drawn as the exanthem appeared. In both instances the diplococci were isolated in anaerobic Krumwiede[11] plain agar plates. without ascitic fluid, as described by Dick and Henry,[12] only 1 or 2 colonies appearing on each plate.

In isolating organisms from the blood of rabbits, plain or dextrose agar plates were made according to the later method of Dick.[13] The whole blood is added to agar melted and cooled to 42 C. and poured into plates in the usual way. The plates are chilled immediately, and as soon as the agar sets another deep tube of sterile agar, melted and cooled to 42 C., is poured over this surface. The plates are again chilled and the surface covered with a layer of melted paraffin 2-3 mm. thick. The paraffin is sterilized by heating to the smoking point and cooled until a film forms on the surface.

Deep tubes of autoclaved 1% dextrose brain broth, preferably with ascitic fluid, described by Rosenow,[14] were also found useful. Chocolate medium and cultures from the blood, laked with sterile distilled water and centrifuged, were not satisfactory. Saponin to destroy the leukocytes as used by Sellards and Bigelow[15] was not tried. On account of their not isolating any diplococci, the conclusion seemed warranted that the saponin might inhibit their growth.

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[11] Ibid., 1913, 12, p. 199.
[12] Ibid., 1914, 15, p. 85.
[13] Ibid., 1918, 23, p. 578.
[14] Arch. Int. Med., 1921, 28, p. 274.

At the last bleeding of an infected rabbit, the citrated blood was centrifuged and cultures made from the top and bottom of the tube, as suggested by Sellards and Bigelow. An abundant growth occurred in ascitic dextrose broth from the top but not from the bottom. No growth was found in cultures of the top fluid when not well diluted, the citrate solution evidently inhibiting growth.

Diplococci similar to those injected were isolated from the Koplik spots and from the blood in 4 of 8 rabbits cultured at the height of the eruption during life and from the lung and blood of 2 other rabbits at necropsy. In 2 cases diplococci were observed in the dextrose brain broth culture, but they would not grow in subculture. One successful culture was taken from a rabbit inoculated with virus, the cocci being isolated from the blood at the height of the eruption on an anaerobic plate and in dextrose brain broth with ascitic fluid. Only one colony appeared on the anaerobic plate, none on the aerobic.

The diplococcus isolated from the lung of the rabbit dying of a bronchopneumonia when injected into another rabbit produced Koplik spots on the third day after injection followed by an eruption on the fifth day.

Two rabbits were injected with the third and fourth generations of the diplococcus isolated during life from the blood of a rabbit inoculated with a diplococcus from the throat of a patient with measles in the pre-eruptive stage. A Koplik spot and eruption was produced in one rabbit on the ninth day and an eruption in a second on the eleventh day. The blood of one of these caused good Koplik spots on the ninth and an eruption on the twelfth day in an inoculated rabbit.

The blood of a rabbit inoculated with a diplococcus was taken at the height of the eruption on the eighth day and inoculated intravenously into 2 rabbits, both of which showed an eruption on the sixth day. The blood of 1 of these was inoculated into another rabbit which developed an exanthem after 3 days' incubation, with a temperature of 105 C. A single colony of green-producing diplococci was isolated from the blood on an anaerobic plain agar plate without ascitic fluid. The experiments indicate that the incubation period is shortened when the organism is passed through animals.

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[15] Jour. Med. Research, 1921, 42, p. 241.

REINOCULATION

One rabbit successfully inoculated with virus and 3 with the diplococcus, on reinoculation 6-10 days later with fresh virus, gave no signs of reaction, while the control rabbits showed distinct rashes, 2 a well marked rise in temperature.

FILTRATION EXPERIMENT

As stated, the green-producing diplococci from measles, when first isolated and grown in medium in which they appear minute such as ascitic dextrose broth and dextrose brain broth, as a rule, pass through tested Berkefeld N filters. As most observers have found that apparently sterile filtrates produce the symptoms of measles in monkeys and rabbits, late subcultures which did not appear to pass through the filter and unfiltered cultures of the same strain and generation were injected intratracheally into 4 rabbits. One rabbit injected with the filtrate showed a Koplik spot on the seventh day, followed by an eruption on the twelfth day; the other 3 rabbits developed rashes—2 on the ninth and 1 on the tenth day. The filtered cultures grossly did not appear to grow, but on careful examination diplococci were demonstrated in smears of the original filtered material and slight growth occurred in subcultures on goat blood, dextrose broth and brain broth. In some instances the diplococci grow out slowly in the original filtrate but multiply more readily on subcultures, especially, if anaerobic.

Rosenow[16] has also observed that green-producing streptococci from influenza may pass through filters, through which B. prodigiosus will not pass, and that they can multiply and grow when injected into the trachea of guinea-pigs and be recovered from the lung, even though the filtered cultures remain negative. Otlitsky[17] has shown that filtrates of typhus virus which are free, so far as they could ascertain from a living, multiplying agent, can occasionally induce in guinea-pigs not only typical lesions of the disease, but also immunity to later injections of the active virus.

SUMMARY

Monkeys, guinea-pigs and rabbits are susceptible to measles when inoculated with the washings of the nose and throat of patients with measles. The same symptoms and lesions may be produced in these animals by the green-producing diplococci isolated from the blood and respiratory passages of human measles.

[16] Jour. Infect. Dis., 1920, 26, p. 504.

[17] Jour. Exper. Med., 1922, 35, p. 469.

Rabbits successfully inoculated with washings or the diplococcus show no symptoms when reinoculated with fresh virus.

Green-producing diplococci from the blood and lung of rabbits, successfully inoculated with diplococci from measles, produce Koplik spots and exanthems when injected into other rabbits.

Filtrates of the diplococcus cultures generally show abundant growth, but old cultures containing large forms sometimes multiply only after several days' incubation or on subculture into a favorable medium.

While the experiments indicate that the reaction is due to the coccus itself and not to a separate virus carried by it, on account of the same symptoms being produced by cultures as late as the seventh generation and the absence of the reaction in rabbits injected with other bacteria, isolated from the same plate as the green producing diplococci, it is possible that the diplococcus possesses the selective power of carrying the specific virus of measles.